

Increased Urinary β_2 -Microglobulin in Cadmium Exposure: Dose-Effect Relationship and Biological Significance of β_2 -Microglobulin

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A study on the general population from both cadmium-polluted and control areas and on cadmium alloy workers indicates that β_2 -MG in urine is very closely correlated with aging, but it indicates an association with cadmium exposure. However, the age factor is stronger than cadmium exposure in both polluted and control areas among persons without clinical proteinuria. On the other hand, cadmium exposure is most likely correlated with β_2 -MG even in nonpolluted areas. Thus it seems that there is no noneffect level of cadmium dose in affecting the elevation of β_2 -MG in urine.

β_2 -MG in serum indicated a very close correlation with cadmium in blood among cadmium alloy workers. This may suggest that an increase of β_2 -MG in both blood and urine in an early stage of cadmium exposure is caused by the increased level of β_2 -MG in blood, which may be a result of stimulation due to cadmium, but not necessarily by the clinical dysfunction of reabsorption of β_2 -MG in the renal tubules.

Introduction

Since itai-itai disease began to be suspected as being related to high cadmium exposure via food, particularly rice, more than ten areas in Japan have been officially designated as being cadmium-polluted. In these areas, some rice samples were found to contain more than 1.0 ppm cadmium. The inhabitants have undergone health examinations conducted by the national or local authorities over a period of several years. The health examinations included qualitative and quantitative determinations of protein in urine and, recently, of β_2 -microglobulin (β_2 -MG). The results as reported in numerous studies have been summarized and reviewed in two monographs (1, 2).

A higher prevalence of increased β_2 -microglobulin in urine among the inhabitants in these

polluted areas has been reported. However, it is known that increased β_2 -microglobulin in urine is also related to the aging process (3, 4). Some studies also discuss the dose-response relationship between β_2 -microglobulin in urine and cadmium exposure in the general environment (3, 5), but in these studies, the dose is not very accurate in quantitative terms because of the length of exposure which covers a period of decades. During this period cadmium concentrations in rice fluctuated greatly; furthermore, the age factor also influences the dose-response relationship between cadmium exposure and β_2 -microglobulinuria. For these reasons, there has been considerable discussion on the dose-response relationship as well as the biological significance of β_2 -microglobulinuria.

The dose-response relationship between cadmium concentration in the air of the working environment and proteinuria in cadmium workers has been discussed by various investigators (1, 2, 6). However, the dose-response relationship between cadmium exposure and β_2 -microglobulin in cadmium-exposed workers is not well documented.

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The relationship between β_2 -microglobulinuria and renal function, specifically the reabsorption in the renal tubuli, has also not been elucidated.

In this paper, cadmium concentration in feces or the total daily excretion of cadmium in feces was used as an indicator of dose to examine the dose-effect relationship between cadmium exposure and β_2 -microglobulin. In examining this relationship, partial correlation coefficients were calculated in order to investigate the influence of age on this dose-effect relationship.

In the second part of this paper, the increase of β_2 -microglobulin in serum of cadmium workers was investigated in relation to increase of cadmium concentration in blood. The relationship between β_2 -microglobulin or cadmium in urine and renal reabsorption function was also examined.

Materials and Methods

Three cadmium-polluted areas and three control areas in Japan were selected for the first part of this study. A total of 216 men, 50 to 70 years of age, who are clinically healthy (in particular, without proteinuria or glucosuria) were the subjects for the study, with about 30 to 40 men from each area. The total daily feces and spot urine samples were collected. Cadmium in feces was analyzed by atomic absorption spectrometry after wet ashing and DDTC-MIBK extraction. Cadmium in urine was measured directly by flameless atomic absorption spectrometry. β_2 -Microglobulin in urine was measured by the radioimmunoassay method.

For the second part of the study, 51 cadmium workers were selected from the rosters of two silver cadmium alloy factories. Cadmium concentration and β_2 -microglobulin in urine were measured, the former by atomic absorption spectrometry after DDTC-MIBK extraction and the latter by radioimmunoassay. In one factory, 18 workers were subjects not only for determinations of cadmium in

urine, but also cadmium in blood. %TRP and %TR β_2 -MG measurement was possible in only 15 workers.

Results

Excretion of Cadmium

Table 1 presents the daily fecal amounts, cadmium in feces, and cadmium and β_2 -microglobulin in urine by area. In Ishikawa Prefecture, daily cad-

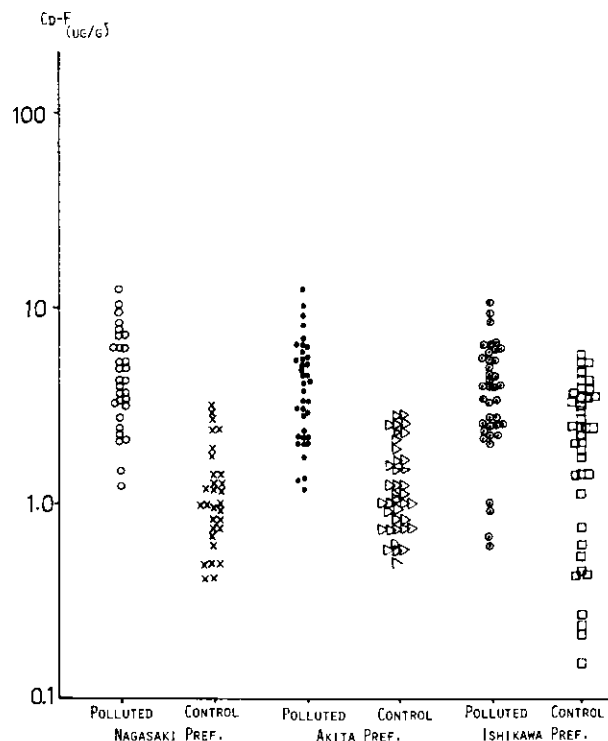


FIGURE 1. Cadmium concentration in feces in polluted and control areas: (O) polluted, Nagasaki Pref.; (X) control, Nagasaki Pref.; (●) polluted, Akita Pref.; (Δ) control, Akita Pref.; (⊙) polluted, Ishikawa Pref.; (□) control, Ishikawa Pref.

Table 1. Daily excretion of cadmium through feces and urine.

	Nagasaki		Ishikawa		Akita	
	Polluted (N = 30)	Control (N = 30)	Polluted (N = 40)	Control (N = 40)	Polluted (N = 37)	Control (N = 37)
Daily fecal amounts, g						
Wet weight	204.3 ± 53.2	174.5 ± 78.2	137.1 ± 87.0	167.5 ± 83.8	183.1 ± 90.5	216.0 ± 110.0
Dry weight	52.6 ± 26.4	34.2 ± 13.3	34.1 ± 21.5	44.7 ± 23.7	38.1 ± 17.3	47.3 ± 24.7
Cd in feces						
ppm	5.04 ± 2.76 ^a	1.27 ± 0.78	4.19 ± 2.30 ^a	2.45 ± 1.66	4.39 ± 2.57 ^a	1.31 ± 0.72
µg/day	255 ± 171 ^a	42.3 ± 30.9	149 ± 121 ^b	102 ± 83.9	158 ± 108 ^a	60.0 ± 49.8
Cd in urine, µg/l.	7.4 ± 4.2	4.8 ± 2.9	10.8 ± 6.1	7.0 ± 5.7	6.1 ± 4.2	
β_2 -MG in urine, mg/g-cr	0.30 3.23	0.11 2.27	0.56 7.31	0.14 2.70	0.16 3.90	

^a Significant, $p \leq 0.01$.

^b Significant, $p \leq 0.05$.

mium in feces was 149 μg in the polluted area and about 102 μg in the control area; thus the difference is in fact not very great. Cadmium concentration in feces (CdF) in polluted areas (Fig. 1) is consistently higher than in the control areas. However, one control area indicated a high CdF with a large standard deviation. Figure 2 shows the daily cadmium excretion in feces; the general trends are similar to those of Figure 1. In one area, the difference between the polluted and control area in Ishikawa Prefecture is very small. The polluted area in Nagasaki Prefecture indicated highest level for cadmium excretion in feces. Cadmium concentration in urine (Fig. 3) also reached higher levels in polluted areas. However, the control area in Ishikawa Prefecture indicated almost the same level as the polluted area in Akita Prefecture. Thus from fecal and urine data, it may be concluded that in the control area in Ishikawa Prefecture, cadmium exposure is somewhat higher than the control areas in other prefectures.

Figure 4 shows β_2 -MG in urine in polluted and control areas. All the polluted areas show higher β_2 -MG levels than the control areas. However, the control area in Ishikawa Prefecture indicates a

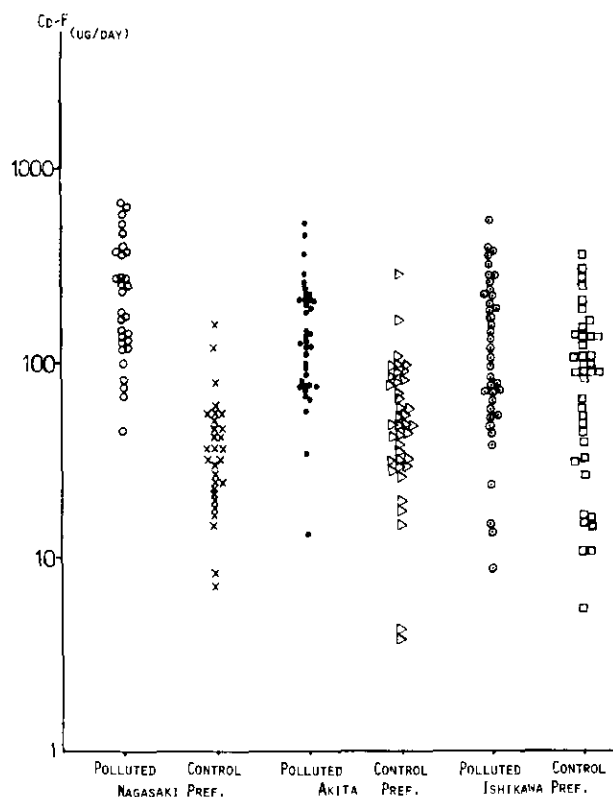


FIGURE 2. Daily cadmium excretion in feces in polluted and control areas. Symbols as in Fig. 1.

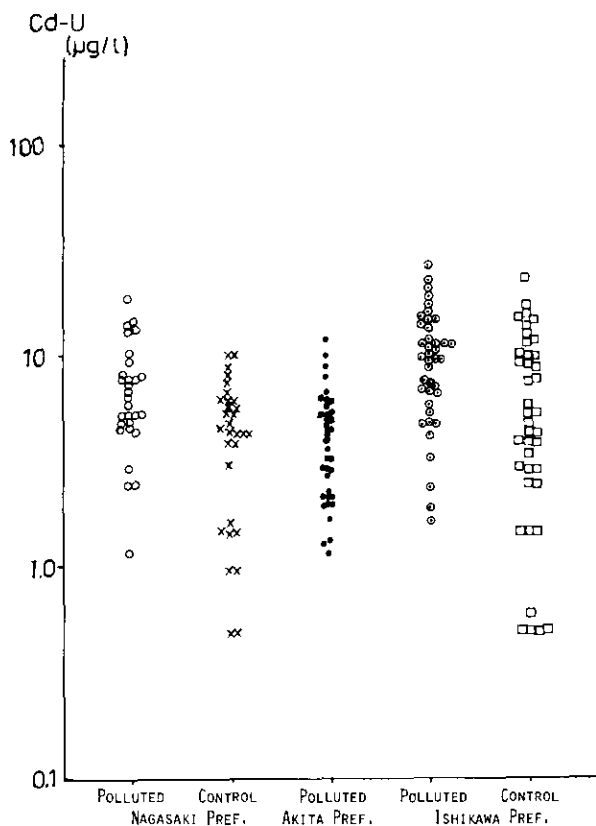


FIGURE 3. Cadmium concentration in urine in polluted and control areas: (○) polluted, Nagasaki Pref.; (X) control, Nagasaki Pref.; (●) polluted, Akita Pref.; (○) polluted, Ishikawa Pref.; (□) control, Ishikawa Pref.

higher β_2 -MG than the control area in Nagasaki Prefecture. This again suggests a higher cadmium exposure in that control area. As shown in Figure 5 (β_2 -MG concentration in urine by age and area), β_2 -MG increases by age in both polluted and control areas. β_2 -MG in urine by daily cadmium excretion and by age is presented in Figure 6. The subjects from the polluted and control areas were combined. The three regression lines were drawn according to grade of cadmium exposure. From 0 to 40 $\mu\text{g/day}$ cadmium excretion in feces is noted by a circle; 60 to 120 $\mu\text{g/day}$ is indicated by a circle with a dot in the center, and 150 $\mu\text{g/day}$ and above is indicated by a black circle. All three regression lines increase with age, but the line for 150 $\mu\text{g/day}$ and over indicates the highest β_2 -MG. However, there is not much difference between the other two regression lines, which also indicate a tendency toward a higher β_2 -MG with age and higher cadmium exposure. The correlation between β_2 -MG and cadmium in urine was significant, as shown in Figure 7. However, the correlation coefficient is somewhat lower than that between β_2 -MG and CdF.

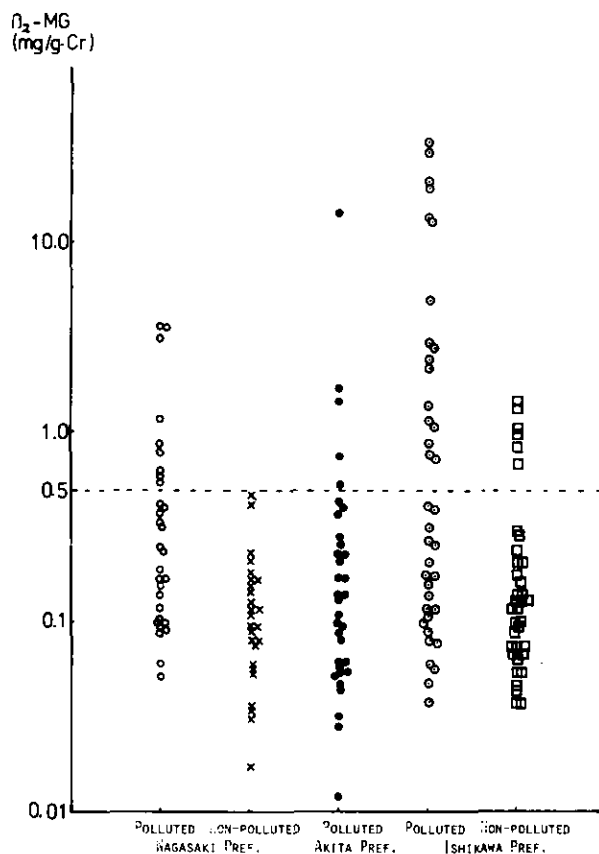


FIGURE 4. β_2 -Microglobulin concentration in urine in polluted and control areas. Symbols as in Fig. 3.

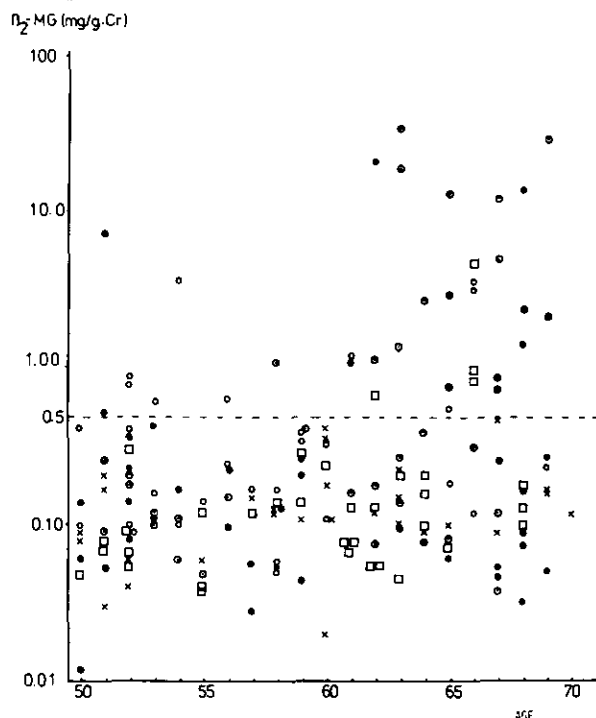


FIGURE 5. β_2 -Microglobulin concentration in urine by age and area. Symbols as in Fig. 3.

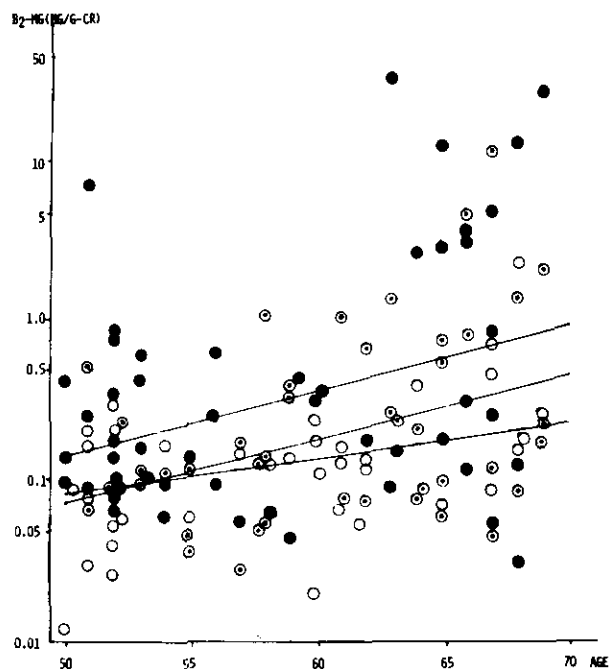


FIGURE 6. β_2 -Microglobulin concentration in urine by daily cadmium excretion: (○) 0-40 $\mu\text{g/day}$; (◐) 60-120 $\mu\text{g/day}$; (●) ≥ 150 $\mu\text{g/day}$.

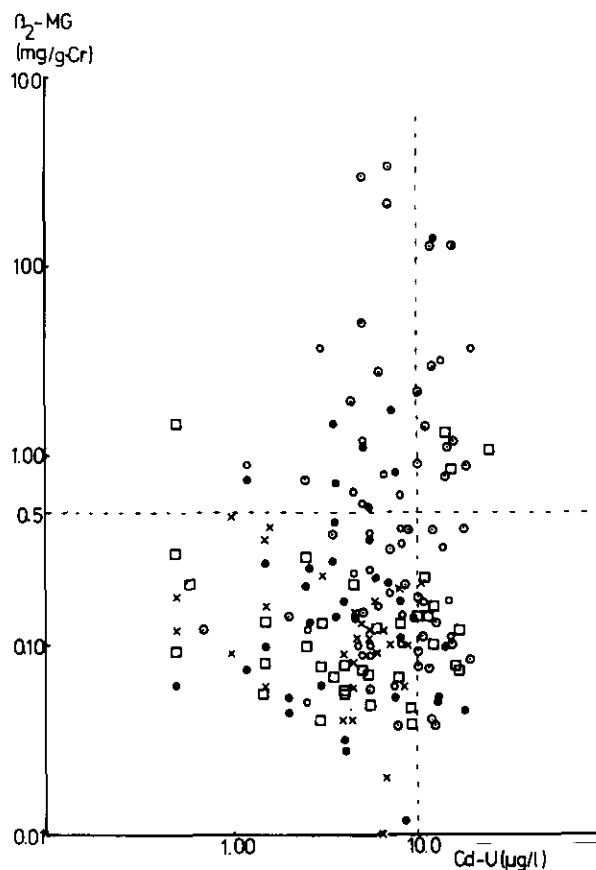


FIGURE 7. Correlation between β_2 -microglobulin and cadmium in urine. Symbols as in Fig. 3.

Table 2. Partial correlation coefficients for β_2 -microglobulin, cadmium in feces, and age.

Correlation ^a	Partial correlation coefficient (simple correlation coefficient)		
	Polluted (n = 107)	Control (n = 70)	Total (n = 177)
$r(\beta_2\text{-MG-CdFD, age})$	0.200 ^b (0.093)	0.072 (0.083)	0.244 ^c (0.196) ^Y
$r(\beta_2\text{-MG-age CdFD})$	0.359 ^c (0.317) ^Y	0.417 ^c (0.415) ^Y	0.360 ^c (0.332) ^Y
$r(\text{age-CdFD, } \beta_2\text{-MG})$	-0.330 ^c (-0.282) ^Y	0.095 (0.043)	-0.177 (-0.101)
$r(\beta_2\text{-MG-CdF, age})$	0.205 ^b (0.154)	0.132 (0.123)	0.282 ^c (0.266) ^Y
$r(\beta_2\text{-MG-age, CdF})$	0.342 ^c (0.317) ^Y	0.418 ^c (0.415) ^Y	0.345 ^c (0.332) ^Y
$r(\text{age-CdF, } \beta_2\text{-MG})$	-0.182 (-0.122)	-0.050 (0.006)	-0.098 (-0.001)

^a β_2 -MG = β_2 -microglobulin; CdFd = amount of Cd total daily in feces; CdF = concentration of Cd in feces.

^b Significant, $p \leq 0.05$.

^c Significant, $p \leq 0.01$.

Table 3. Partial correlation coefficients for β_2 -MG and Cd in urine and age.

Correlation ^a	Partial correlation coefficient (simple correlation coefficient)		
	Polluted	Control	Total
$r(\beta_2\text{-MG-CdU, age})$	0.064 (0.103)	0.1419 (0.137)	0.132 (0.161) ^Y
$r(\beta_2\text{-MG-age, CdU})$	0.3077 ^c (0.317) ^Y	-0.0420 (0.019)	0.320 ^c (0.322) ^Y
$r(\text{CdU-age, } \beta_2\text{-MG})$	0.1085 (0.135)	0.4164 ^c (0.415) ^Y	0.0657 (0.113)

^a CdU = Cd concentration in urine.

^b Significant, $p \leq 0.05$.

^c Significant, $p \leq 0.01$.

Tables 2 and 3 indicate partial correlation coefficients among three factors, namely, for β_2 -MG in urine, cadmium in feces, and age. In both polluted and control areas, although there are significant correlations between β_2 -MG and daily CdF, the correlation between β_2 -MG and age is consistently much higher than that between β_2 -MG and daily CdF. It is also important to note here that even in control areas there is a tendency toward a correlation between β_2 -MG and CdF at constant age. This clearly indicates that both cadmium exposure and age are highly involved in the increase of β_2 -MG, with age consistently being the stronger influencing factor. In addition, it seems that there is no no-effect level of cadmium exposure for influencing β_2 -MG level.

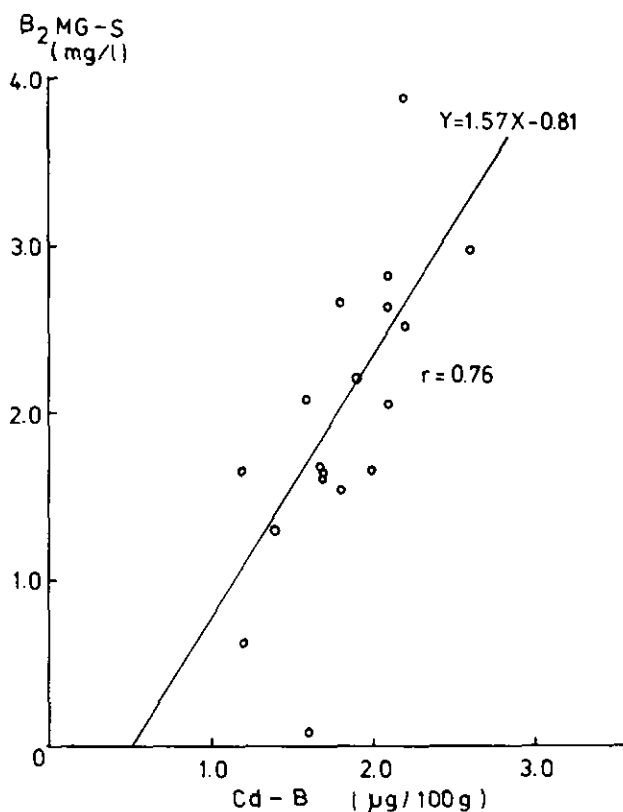


FIGURE 8. Correlation between cadmium and β_2 -microglobulin in blood.

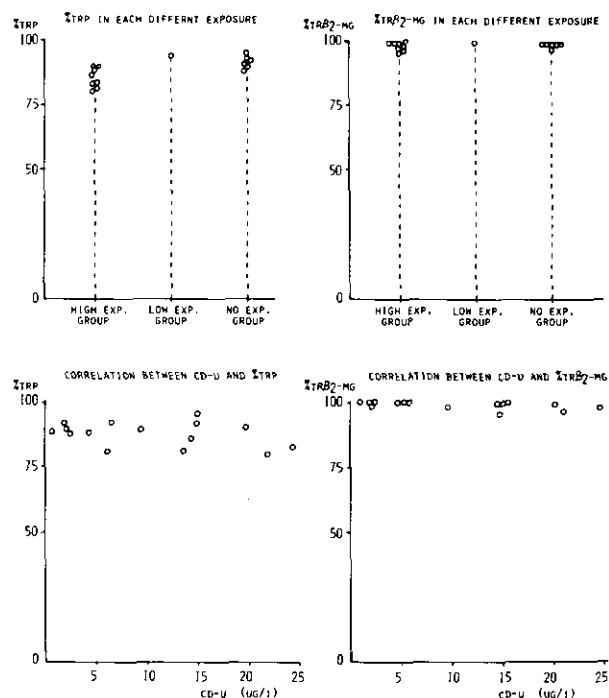


FIGURE 9. Associations between cadmium exposure and renal tubular functions or β_2 -microglobulin.

Cadmium in Blood in Cadmium Workers

Figure 8 indicates an association between β_2 -MG in serum and cadmium concentration in blood of 18 cadmium workers from a silver cadmium alloy factory. As shown in Figure 8, there is a high significant correlation ($r = 0.76$) between the two factors. This suggests that β_2 -MG in blood increases with increased cadmium in blood. Figure 9, includes four plots, showing the association between %TRP, %TR β_2 -MG, cadmium exposure, and cadmium concentration in urine of 15 workers. As shown in these figures, renal tubular function, represented by %TRP or %TR β_2 -MG, is not lowered by increased cadmium exposure in this case. The highest cadmium excretion in urine, however, was only 25 $\mu\text{g/l}$.

Discussion

Over the years, a number of papers have been published on proteinuria among cadmium workers and it has been well established as an early sign of chronic cadmium poisoning (1, 2). However, the quantitative determination of β_2 -MG, which is the main component in proteinuria due to cadmium poisoning in man, has not been well documented, especially in terms of dose-response relationships. Some reports on inhabitants from cadmium-polluted areas in Japan indicated a clear-cut dose-effect relationship between, for example, years of residence in a polluted area and increased excretion of β_2 -MG (3, 5). However, some other reports have failed to observe a higher rate of increased β_2 -MG in cadmium-polluted areas compared to control areas (2). Furthermore, some reports (3, 4) reported a close association between increase of β_2 -MG and age.

In the reports published up to the present, there are two problems: one is the need for more exact determination of dose or exposure than length of years of residence in a polluted area; the other is to separate the two factors of cadmium exposure and age by more sophisticated statistical analysis. The first part of this study was performed to help solve these problems.

Increase of β_2 -MG in urine at an early stage of cadmium exposure has been demonstrated to be more sensitive than proteinuria by sulfosalicylic acid (7). However, the clinical significance of increased β_2 -MG in urine at an early stage of exposure remains a question, specifically in relation to clinical renal tubular dysfunction. The second part of this paper is concerned with the mechanism of the increase of β_2 -MG before the development of clinical renal tubular dysfunction.

In the first part of this study, cadmium concen-

tration or daily cadmium excretion of feces was used as the indicator of cadmium exposure in polluted areas where inhabitants are exposed to excess cadmium via food, particularly rice. It would seem that cadmium in feces could reflect the present condition of cadmium exposure via food. However, because of the control measures against cadmium pollution and excess intake of cadmium taken by the government, cadmium in feces measured in the present study may not necessarily represent the grade of past exposure. Further, since feces samples were collected over 24-hr periods, they may not necessarily represent average cadmium excretion over a long period in an individual. Nevertheless, it can be safely stated that daily excretion of cadmium is the best direct indicator of exposure in practical terms.

In fact, the present study did reveal an association between cadmium in feces and β_2 -MG in urine. But at the same time, there was an even higher partial correlation coefficient between age and β_2 -MG when cadmium in feces was fixed. This was even more clear in the control areas. In the control areas, however, an almost significant correlation between cadmium in feces and β_2 -MG was observed when age was fixed. These findings clearly indicate that both cadmium in feces and age are closely associated with β_2 -MG in a population without quantitative proteinuria. In such a population, age is more strongly associated with β_2 -MG than cadmium exposure. However, the grade of exposure seemed to be associated with β_2 -MG increase even among a population with a "normal" exposure to cadmium via food. This may well suggest that there is no noneffect level of cadmium exposure for increase of β_2 -MG. It is also important to point out, based on the findings in this study, that in a study on β_2 -MG on the general population, the age factor should not be ignored. For instance, when length of residence is used as an index of exposure, the age factor should be fixed in statistical analysis.

In the second part of this study, the investigation of cadmium alloy workers, a high association was observed between cadmium in blood and β_2 -MG in serum. This may suggest that cadmium is a direct stimulant for the production of β_2 -MG in blood. In fact, Harada (8) recently reported a similar result. In the same study, Harada also observed an increase of β_2 -MG in urine only three months after exposure to cadmium among cadmium workers. Furthermore, no associations between the grade of exposure to cadmium or cadmium concentration in urine, up to 25 $\mu\text{g/l}$ cadmium, and %TRP or %TR β_2 -MG were recognized among cadmium alloy workers. This indicates that these workers do not suffer clinical dysfunction of renal tubular reab-

sorption, in spite of increased β_2 -MG in urine.

According to these findings, it is difficult to assume the development of clinical renal tubular dysfunction which leads to an elevated β_2 -MG in urine at an early stage of cadmium exposure. Thus it seems that an increase of β_2 -MG at an early stage of exposure is caused by a mechanism other than renal tubular dysfunction, most likely increased β_2 -MG in blood, which results in an increase of β_2 -MG in urine.

Conclusion

According to the present study on the general population from both cadmium-polluted and control areas and on cadmium alloy workers, the following conclusions were drawn.

β_2 -MG in urine is very closely correlated with aging, but it indicates an association with cadmium exposure. However, the age factor is stronger than cadmium exposure in both polluted and control areas among persons without clinical proteinuria. On the other hand, cadmium exposure is most likely correlated with β_2 -MG even in nonpolluted areas. Thus it seems that there is no noneffect level of cadmium dose in affecting the elevation of β_2 -MG in urine.

β_2 -MG in serum indicated a very close correlation

with cadmium in blood among cadmium alloy workers. This may suggest that an increase of β_2 -MG in both blood and urine in an early stage of cadmium exposure is caused by the increased level of β_2 -MG in blood, which may be a result of stimulation due to cadmium, but not necessarily by the clinical dysfunction of reabsorption of β_2 -MG in the renal tubules.

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